Characterization and Localization of the Rabbit Ocular Calcitonin Gene-Related Peptide (CGRP)-Receptor Component Protein (RCP)

Mark I. Rosenblatt,1 Gerhard P. Dahl,2 and Ian M. Dickerson1,2

PURPOSE. To determine whether the calcitonin gene-related peptide (CGRP) receptor component protein (RCP), a novel signal transduction molecule, is required for CGRP signaling in the eye and to determine potential ocular sites of CGRP action.

METHODS. The cDNA for the rabbit ocular RCP homologue was cloned using a combination of reverse transcription-polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends (RACE). Function of the rabbit ocular RCP was assessed using a sensitive oocyte-based assay, which utilizes the protein kinase A (PKA)-sensitive cystic fibrosis transmembrane conductance regulator (CFTR) as a sensor of cAMP formation. RCP expression in the rabbit eye was localized using immunohistochemistry.

RESULTS. A 2063-bp cDNA for the rabbit ocular RCP was cloned and sequenced. Expression of the rabbit RCP cDNA confers CGRP responsiveness in a sensitive oocyte-based assay. Antisense oligonucleotides made to the ocular RCP abolishes CGRP responsiveness of ciliary body and iris mRNA in the oocyte-CFTR assay. Localization of RCP protein in the rabbit eye using immunohistochemistry demonstrated RCP immunoreactivity in the ciliary body and iris blood vessels, as well as in layers of the ciliary epithelium.

CONCLUSIONS. The rabbit ocular RCP appears to be required for signal transduction at ocular CGRP receptors and is localized to sites previously reported to bind CGRP, which affect intraocular pressure and neurogenic inflammation. (Invest Ophthalmol Vis Sci. 2000;41:1159–1167)

The neurogenic inflammatory response to ocular trauma is characterized by increased anterior uveal blood flow, elevated intraocular pressure, a breakdown of the blood-aqueous barrier, increased cAMP levels in the aqueous humor, and miosis.1–5 This ocular neurogenic inflammatory response is initiated by traumatic insult to the eye with resultant antidromic stimulation of sensory neurons, leading to the release of inflammatory mediators into the anterior uvea.4,5 Calcitonin gene-related peptide (CGRP), a 37-amino acid neuropeptide that has potent vasodilator action,6 has been implicated as a mediator of this neurogenic inflammatory response.7–9 In the eye, CGRP has been colocalized with substance P (SP) in neurons that ensheathe the blood vessels of the anterior uvea.10,11 CGRP levels increase in the aqueous humor after ocular trauma and initiation of a neurogenic inflammatory response.12 Furthermore, intracameral administration of CGRP mimics the increased blood flow, elevated intraocular pressure, and increased cAMP levels associated with neurogenic inflammation.7–9,13–15 In support of the role for CGRP action in the ciliary body and iris, CGRP binding sites are present in ciliary process membranes16 and 125I-CGRP binding in ocular tissue sections has demonstrated the presence of CGRP binding sites in the anterior uvea.17 Together, these results suggest the presence of CGRP and CGRP receptors in the anterior eye, which are responsible for mediating an inflammatory response in the eye.

We recently described the CGRP-receptor component protein (RCP) as a novel molecule required for CGRP signal transduction.18,19 RCP is a novel hydrophilic 146-amino acid protein, which is colocalized with CGRP immunostaining neurons, and is required for CGRP receptor activity in cerebellum and cochlea.18 Additionally, expression of RCP correlates with CGRP function in the pregnant mouse uterus, where lowered levels of RCP protein paralleled reduced inhibitory effects of CGRP on myometrial contraction.19 Because of its small size and hydrophilic nature, we do not think that RCP represents a CGRP receptor itself and instead hypothesize that RCP works in conjunction with a membrane-spanning protein to form a functional CGRP receptor complex. CGRP receptors thus represent one of the first examples of G protein–coupled receptors that require an accessory protein. Here, we determined that CGRP receptors in the eye share a similar requirement for RCP by cloning a cDNA for RCP from the rabbit eye, demonstrating that this cDNA confers CGRP responsiveness in an oocyte-cystic fibrosis transmembrane conductance regulator (CFTR) assay and localizing RCP expression to potential sites of CGRP action in the eye.
METHODS

Animal Care and Tissue Extraction

Animals were cared for in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Male New Zealand albino rabbits were killed by intravenous injection of pentobarbital. Eyes were immediately enucleated and transferred to 10 mM phosphate-buffered saline (PBS), pH 7.4. For RNA extraction, the anterior eye was removed, and the ciliary body and iris were microdissected. For immunohistochemistry, eyes were cut tangentially and washed immediately with 10 mM PBS.

RNA Extraction

Total RNA was isolated from freshly dissected ciliary body and iris, which was homogenized in Tri-Reagent (Molecular Research, Cincinnati, OH) using a Polytron tissue homogenizer (Polytron; Brinkman, Westbury, NY). Total RNA was purified from the homogenate according to the manufacturer’s instructions. Poly(A) RNA was isolated from the total RNA using the PolyATtract mRNA System (Promega, Madison, WI).

Isolation of a Rabbit RCP Amplimer by Reverse Transcription–Polymerase Chain Reaction

A portion of the rabbit RCP cDNA was isolated using degenerate reverse transcription–polymerase chain reaction (RT-PCR).19 RT of rabbit ciliary body and iris mRNA (150 ng) was primed using the primer DRES-1 (5’ TCY TGY ATN ACC ATN ARY TGN ATC YTY TGN ACN 3’; IUPAC codes where Y = C or T, R = A or G, and N = any), a degenerate oligonucleotide primer designed to regions conserved between the guinea pig and rabbit ciliary body and iris mRNA using an oligo-dT primer. Second-strand cDNA was synthesized using standard conditions (Clontech). After blunting of the double-stranded cDNA with T4 DNA polymerase, adapters (Clontech) were ligated to the cDNA ends using T4 DNA ligase.

The adapter-ligated cDNA was used as a template for 5’ and 3’ RACE reactions using oligonucleotide primers designed to the rabbit RCP fragment obtained by RT-PCR. A downstream primer (RRES-4: 5’ GAC AAT CTC AGG ACT CTG GTG CCG ACA TGG 3’) and adapter primer-1 (AP-1) were used for 5’ RACE reactions and an upstream primer (RRES-3: 5’ ACA AAG TGG GAA GAA TAA ACA GAG TTC TGG 3’) and adapter primer AP-1 were used for 3’ RACE reactions (Fig. 1). RACE reactions were performed using the Expand PCR Kit (Boehringer-Mannheim, Indianapolis, IN). Initial 5’ RACE products were reamplified using a downstream primer internal to RRES-4 (RRES-6: 5’ CCT ATG AAA CAT TAA AGT ACA TAT CAA AAA 3’) and an adapter primer (AP-2) internal to AP-1. Initial 3’ RACE products were reamplified using a nested upstream primer (RRES-5: 5’ GGC AAC AGA AGT TAGA CTA TCA TGA CC 3’) and adapter primer AP-2. Primers RRES-5, RRES-6, and AP-2 contained 12-nucleotide additions at their 5’ ends, allowing final 5’ and 3’ RACE products to be cloned using the CloneAmp System (GIBCO-BRL). RACE clones were sequenced using dideoxy double-stranded thermal cycle sequencing (AmpliCycle Sequencing Kit; Perkin-Elmer).

Expression of the Cloned Rabbit RCP

Primers RRES-10 (5’ ATC TGC AGG CTG CTG CCG AC 3’) and RRES-11 (5’ GTG CAT CTG GCC CAG CTG TTT TAT GGA ATT CG 3’) were designed to flank the rabbit RCP open reading frame (ORF) (Fig. 1A). Ciliary body and iris mRNA (150 ng) was reverse-transcribed using RRES-10. First-strand cDNA was subsequently amplified by PCR using RRES-9 and RRES-10. Primers were designed with restriction sites (RRES-10: Psfl and RRES-11: EcoRI), which allowed ligation of the PCR amplimer into the low background vector, pZero (Invitrogen, Carlsbad, CA), resulting in the plasmid pRCP.Zero. The ligation product was transformed into Escherichia coli (One Shot Top10F; Invitrogen), and the transformed E. coli was grown in liquid culture in media containing 1 mM IPTG to lower non–insert-containing plasmids, as described by the manufacturer. Plasmid DNA was isolated and tested using the oocyte–CFTR assay.

Oocyte–CFTR Assay

Follicular cells were removed and Xenopus laevis oocytes were prepared for injection as described.21 Oocytes were coinjected with 50 ng of CFTR cRNA and either 15 ng ciliary body and iris mRNA or 50 ng rabbit RCP cRNA. The CFTR cDNA (pACF2 obtained from J. Riordan, Mayo Clinic, Scottsdale, AZ) and rabbit RCP (pRCP.Zero) were transcribed in vitro using the mMessage mMachine Kit (Ambion, Austin, TX) to synthesize cRNA for injection. For antisense experiments, 50 ng of phosphorothioate oligonucleotide to the rabbit RCP (RP-CAPAS3: 5’ GCA GCC TCC TCT CGT AGG T 3’) or rabbit calcitonin receptor (RP-CAS3: 5’ TGG AGT AGA TTG CTC GAG TAA TAG GTG G 3’) were coinjected with RNA. After a 48- to 72-hour incubation to allow expression, oocytes were voltage clamped at −50 mV and incubated with 100 nM CGRP (Bachem Bioscience, King of Prussia, PA), as previously described.18 Maximum cAMP-induced CFTR response was determined by incubation with 20 μM forskolin (Sigma, St. Louis, MO).

Immunohistochemistry

Freshly prepared eyes were fixed for 2 hours in 4% paraformaldehyde in 0.01 M PBS. Fixed eyes were passed through a graded series of ethanol washes (0%, 70%, 90%, 100%) and paraffin embedded. Eight-micrometer sections were mounted
on 3-triethoxysilylpropylamine (TESPA)-coated slides (Sigma, St. Louis, MO). Sections were incubated in 100% xylene for 10 minutes and rehydrated through a graded series of ethanol: 100%, 90%, 70%, 0%. Sections were washed 10 minutes in 0.01 M PBS and treated 15 minutes in 10% methanol/3% hydrogen peroxide. After a 10-minute wash in 0.01 M PBS, sections were blocked for 30 minutes in 0.01 M PBS containing 5% normal horse serum (Hyclone, Logan, UT) and 0.01% Triton X-100. The blocking solution was removed and sections were incubated in primary (immune or pre-immune) antibody 1066 (1:1500 dilution) against a synthetic peptide (EEQIEALLHTVT) conjugated to keyhole limpet hemocyanin. Sections were washed in PBS, and rehydrated through a graded series of ethanol: 100%, 90%, 70%, 0%. Sections were incubated in primary antibody 1066 (1:1500 dilution) against a synthetic peptide (EEQIEALLHTVT) conjugated to keyhole limpet hemocyanin. Sections were washed in PBS, and rehydrated through a graded series of ethanol: 100%, 90%, 70%, 0%. Sections were incubated in primary antibody 1066 (1:1500 dilution) against a synthetic peptide (EEQIEALLHTVT) conjugated to keyhole limpet hemocyanin. Sections were washed in PBS, and rehydrated through a graded series of ethanol: 100%, 90%, 70%, 0%. Sections were incubated in primary antibody 1066 (1:1500 dilution) against a synthetic peptide (EEQIEALLHTVT) conjugated to keyhole limpet hemocyanin.
fixed 20 minutes in 0.1% glutaraldehyde, washed again in PBS, and incubated with a 1:400 dilution of biotin-conjugated donkey anti-chicken secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA) in 10 mM PBS. Immune complexes were detected using avidin–biotin complex reagent coupled to horseradish peroxidase (ABC Reagent; Vector Laboratories, Burlingame, CA) and visualized by light microscopy and images collected on a Pixera digital camera (Pixera Corporation, Los Gatos, CA).

RESULTS

Cloning of the Rabbit RCP from the Ciliary Body/Iris

We previously demonstrated that the CGRP receptor signaling in the cochlea and uterus is dependent on RCP.18,19 To test our hypothesis that RCP is similarly required in the eye for CGRP-mediated signal transduction, a cDNA encoding the rabbit ocular RCP cDNA was isolated for testing in an oocyte-based assay. A 206-bp fragment of the rabbit RCP ORF was amplified by RT-PCR from ciliary body and iris mRNA using degenerate primers (Fig. 1A) designed to regions of protein homology between the guinea pig and mouse RCP. Sequencing indicated that the resulting amplimer shared a high degree of homology with the guinea pig and mouse RCP and coded for a fragment of the rabbit RCP homologue (Figs. 1A, 1B).

The sequence obtained from the RT-PCR amplimer was used to design specific primers to the rabbit RCP to facilitate amplification of the 5′ and 3′ ends of the rabbit ocular RCP cDNA by RACE. Overlapping RACE amplimers were obtained, and a 200-bp 5′ RACE clone and an 1100-bp 3′ RACE clone were isolated and sequenced. The rabbit RCP cDNA contains a 444-bp ORF coding for a 146-amino acid protein (Fig. 1A). The rabbit ocular RCP cDNA contains a consensus Kozak translation initiation site and an in-frame stop codon 72 bp upstream from the initiator methionine in the 5′ untranslated region. The rabbit RCP does not contain any sites for N-linked glycosylation but does contain several consensus phosphorylation sites that are conserved between guinea pig, mouse, and human (Fig. 1B). The rabbit RCP protein is homologous to previous cloned RCPs (Fig. 1B) sharing 81.5%, 87.1%, and 91.2% identity to the guinea pig, mouse, and human RCP, respectively. Similar to the guinea pig, mouse, and human homologues, the rabbit RCP is hydrophilic and contains no identifiable signal peptide sequence.

Functional Expression of the Rabbit Ocular RCP cDNA

The role of ocular RCP in CGRP-mediated signal transduction was determined using an oocyte-based assay originally described by Uezono et al.24 In this assay the CFTR was used as a sensor for cAMP-mediated signal transduction. Elevated cAMP levels activate PKA, which in turn activates the CFTR, causing this chloride channel to open and produces a measurable inward current. We have previously demonstrated that RCP from cochlea and uterus confers responsiveness to CGRP using this oocyte–CFTR assay.18,19 Xenopus are known to express CGRP receptors,24,25 which were presumed to be inactive in oocytes without the addition of RCP. We have not observed endogenous CGRP receptor activity in CFTR-injected oocytes without the addition of RCP.

The oocyte–CFTR assay was used to test whether the ocular RCP could confer CGRP responsiveness. The rabbit RCP ORF was amplified from ciliary body and iris mRNA using RT-PCR, and the resulting amplimer ligated into the plasmid, pZero (Invitrogen). Plasmid DNA was linearized and transcribed in vitro, the cRNA was coinjected with CFTR cRNA into oocytes, and RCP function (CGRP responsiveness) was tested using the oocyte–CFTR assay as described16,19 (Fig. 2A). Incubation of oocytes coinjected with rabbit RCP cRNA and CFTR cRNA with 100 nM CGRP resulted in CFTR currents of 400 nA, indicating that the rabbit RCP could confer CGRP responsiveness to oocytes (Fig. 2B). Oocytes injected with CFTR cRNA alone did not respond to CGRP (Fig. 2B) but did respond to forskolin (data not shown).

RCP Antisense Experiments

Our model predicts that RCP in the eye is required for signal transduction at CGRP receptors. To provide further evidence for the requirement of RCP in CGRP signal transduction in the rabbit ciliary body and iris, we evaluated the ability of antisense oligonucleotides made to the cloned rabbit ocular RCP to inhibit the activity of rabbit ciliary body and iris mRNA in the oocyte–CFTR assay. Messenger RNA isolated from ciliary body and iris conferred CGRP responsiveness in the oocyte–CFTR assay when coinjected with CFTR cRNA (Fig. 3A), similar to the effect observed for RCP cRNA described above. These results indicate that the ciliary body and iris expresses mRNA for a protein or proteins required for responsiveness to CGRP in oocytes. To determine whether RCP is responsible for the CGRP responsiveness conferred by ciliary body and iris mRNA in the oocyte–CFTR assay, antisense oligonucleotides designed against the RCP cDNA were coinjected with the CFTR cRNA and ciliary body and iris mRNA to deplete RCP mRNA from the pool of ciliary body and iris mRNA. Although oocytes injected with mRNA and CFTR cRNA responded to 100 nM CGRP (Fig 3A), the addition of RCP antisense oligonucleotides abolished the CGRP-induced currents (Fig. 3B). Injection of unrelated antisense oligonucleotides (to the calcitonin receptor) had no effect on CGRP-induced currents (Fig. 3C), indicating that the loss of signal associated with the RCP antisense oligonucleotides was specific for the CGRP receptor and not due to nonspecific effects of phosphorothioate oligonucleotides. Data for four such experiments (four different oocytes) are shown in Figure 3D. These experiments demonstrated that the RCP mRNA contained within the ciliary body and iris mRNA is a requisite factor for CGRP signal transduction in the oocyte.

Immunohistochemical Localization or RCP in the Rabbit Eye

Given the ability of CGRP to augment ocular blood flow, increase intraocular pressure and cause breakdown of the blood–aqueous barrier, CGRP most likely acts on the ciliary body and iris blood vessels that determine blood flow. CGRP may influence aqueous humor dynamics as well by acting on the ciliary epithelium, which is responsible for active production of aqueous humor and maintenance of the blood–aqueous barrier. If RCP were required for CGRP signal transduction in the ciliary body and iris, then RCP would be expected to be in close association with CGRP binding sites. RCP protein expres-
sion in the eye was determined by immunohistochemistry, using a polyclonal antibody 1066 raised in chicken against a synthetic peptide to RCP. Dense immunostaining for RCP was observed in the ciliary processes along the ciliary epithelial layer as well as blood vessels within the stroma of the ciliary processes (Figs. 4A, 4B). Within the iris, the posterior epithelium is densely stained as well as blood vessels of varying caliber that are close to the epithelium (Figs. 4D, 4E). It appears that the nonpigmented layer of the ciliary epithelium and the most superficial layer of the iris posterior epithelium stain most densely for RCP expression (Fig. 4). The iris dilator muscle, anterior to the iris epithelium, also expresses RCP. Immunohistochemistry with preimmune serum showed no staining, indicating that the immunoreactivity seen in the ciliary processes and iris is specific for RCP (Figs. 4C, 4F).

Expression of RCP protein in the rabbit lens and retina also was examined. RCP staining was observed in the lateral lens fibers, but not in the most peripheral portions of the lens or in the lens epithelial layer (Figs. 5A, 5B). Immunoreactive regions of the retina included the retinal ganglion cells and cells within the amacrine layer as well as diffuse staining of the choroid, with more intense staining of the choroidal blood vessels (Figs. 5D, 5E). Preimmune serum failed to stain either the lens or retina, confirming the specificity of the RCP localization (Figs. 5C, 5F).
DISCUSSION

CGRP has been well characterized as a mediator of the ocular neurogenic inflammatory response to injury, but the localization of CGRP receptors and the mechanisms by which CGRP causes this inflammation are not well understood. Studies on the role of CGRP in the physiology of the eye would be facilitated by the molecular identification of the CGRP receptor. However, the CGRP receptor has been difficult to identify, in part because the CGRP receptor appears to be one of the first examples of a G protein–coupled receptor that requires an accessory protein for function. This has complicated the isolation of the receptor cDNA and subsequent reconstitution of CGRP receptor activity by transfection in cell culture. We discovered an accessory protein for CGRP receptor activity as a result of expression cloning studies aimed at cloning the CGRP receptor using an oocyte-based assay. We cloned a cDNA that encoded a small hydrophilic protein named the CGRP-receptor component protein (RCP), which conferred CGRP responsiveness to Xenopus oocytes and is expressed in parallel with CGRP biological activity. RCP is not a receptor itself, but we hypothesize instead that RCP works in conjunction with a membrane-spanning receptor to constitute a functional CGRP receptor complex.

To better understand the role of CGRP in ocular pathophysiology, we have cloned and characterized rabbit ocular RCP. Our hypothesis is that the ocular RCP also is necessary for signal transduction at CGRP receptors in the eye and is colocalized with CGRP receptor sites in the ciliary body and iris. The full-length rabbit RCP cDNA was cloned from the ciliary body and iris using a combination RT-PCR and RACE. Like the guinea pig and mouse RCP, the 148-amino acid rabbit RCP is hydrophilic, contains no identifiable signal peptide, and is not homologous to any other known signal transduction molecule. The cloned rabbit ocular RCP was sufficient to confer responsiveness to CGRP when expressed in the oocyte–CFTR assay, presumably working in conjunction with an endogenous CGRP receptor expressed in Xenopus, which is inactive without the addition of RCP. CGRP responsiveness also was conferred in the oocyte–CFTR assay by coinjection of eye mRNA with CFTR cRNA. This activity was abolished by coinjection of RCP antisense oligonucleotides, indicating that the CGRP receptor activity conferred by the eye mRNA required RCP for function. Antisense oligonucleotides to RCP did not affect the responsiveness of oocytes to forskolin, indicating that the antisense oligonucleotides did not have a general inhibitory effect on cAMP-mediated signal transduction. These data suggest that the rabbit RCP is a component of a CGRP signal transduction pathway in the eye.

Previous studies have demonstrated CGRP binding sites in the eye using autoradiographic and membrane binding techniques. Heino et al. have reported I-CGRP binding in the ciliary processes, iris, ciliary muscle, chamber angle, limbal conjunctiva, choroidae, and retina. Using immunohistochemistry, we found a correlation between RCP expression and these sites previously reported to bind CGRP. Our immunohistochemical results indicate that RCP is localized to numerous blood vessels in the iris as well as to the epithelial layers coating the ciliary body, which are chiefly responsible for the active secretion of aqueous humor. The vascular localization of the RCP is consistent with the augmentation of blood flow by CGRP seen in the neurogenic inflammatory response and

FIGURE 3. RCP is required for CGRP receptor function in ciliary body and iris. Messenger RNA from ciliary body and iris was coinjected with CFTR cRNA and either no antisense oligonucleotides, antisense oligonucleotides to RCP (anti-RCP) (A), or antisense oligonucleotides to a control mRNA (anti-control; calcitonin receptor) (B). Oocytes were voltage-clamped, and responses to 100 nM CGRP were determined. Results from multiple experiments with no antisense oligonucleotides and anti-RCP oligonucleotides are shown in (C). Data from separate oocytes (n = 4) were used to determine the current generated by CGRP as a percentage of the maximal current induced by 20 nM forskolin (% Max). Error bars, SEM. *Statistically significant (Student's t-test) with P < 0.001. For all traces, the upward arrow indicates addition of CGRP and the downward arrow indicates washout. Inward current is indicated by upward deflection in the current trace.
correlates with the distribution of CGRP-immunoreactive nerve fibers around blood vessels in the iris and ciliary body.\textsuperscript{10,11,28} RCP staining of the ciliary epithelial layers also may explain CGRP-induced elevation of intraocular pressure and breakdown of the blood-aqueous barrier.\textsuperscript{2,8,9,13,15,29–32}

The nerve fibers that release CGRP are in close proximity with

---

**Figure 4.** RCP localization in the ciliary processes and iris. Immunohistochemistry was performed with antibody 1066 on 8-\(\mu\)m rabbit eye sections. Ciliary process immunoreactivity was visualized at low (A) and high (B) power. Iris staining was visualized at low (D) and high (E) power. Control experiments for ciliary processes (C) and iris (F) were performed with preimmune 1066 serum at low power. Scale bars, (A, C, D, F) 100 \(\mu\)m; (B, E) 30 \(\mu\)m.

**Figure 5.** RCP localization in the lens and retina. Immunohistochemistry was performed with antibody 1066 on 8-\(\mu\)m rabbit eye sections. Lens immunoreactivity was visualized at low (A) and high (B) power. Retina staining was visualized at low (D) and high (E) power. Control experiments for lens (C) and retina (F) were performed with pre-immune 1066 serum at low power. Scale bars, (A, C, D, F) 100 \(\mu\)m; (B, E) 30 \(\mu\)m.
the ciliary epithelial layers, and CGRP once released may diffuse to these sites. It has been established that agents such as CGRP that increase cAMP levels can increase the active secretion of aqueous humor by the ciliary epithelium, resulting in elevated intraocular pressure. The tight junctions between cells of the nonpigmented epithelium are the anatomic sites of the blood–aqueous barrier, so that CGRP may be directly acting on these cells to cause disruption of the blood–aqueous barrier.

RCP immunoreactivity also was seen in the lens and retina. Within the lens, RCP staining was evident in lateral lens fibers but not in lens fibers closest to the lens surface or in the lens epithelium. CGRP is a growth factor in several systems, and the patterned distribution of RCP in the lens suggests a developmentally regulated expression of RCP, although no role for CGRP in lens development has been studied. This lens localization is also consistent with the isolation of an expressed sequence tag for an RCP homologue (GenBank accession no. D26513) from a chick lens cDNA library. In the retina, immunoreactive RCP was most apparent in the Müller cells and in cells of the amacrine layer. CGRP has been reported to cause subtle changes in the rabbit electroretinogram, which is consistent with the localization of RCP in the retinal amacrine layer. RCP also is expressed by the vascular choroidal layer of the retina, suggesting a possible role for CGRP in regulating retinal blood flow.

A candidate CGRP receptor named the calcitonin receptor-like receptor (CRLR) was previously cloned by a PCR-based approach, but did not initially confer CGRP receptor activity when transfected into cell culture. Subsequently cloned a receptor they named CGRP1, which was identical with CRLR and was active when stably transfected into HEK293 cells but not in COS cells, leading the authors and others to suggest the requirement for a second protein for CRLR function. Although cotransfection of CRLR with RCP into COS cells has not yielded functional CGRP receptors (Rosenblatt MI, Dickerson IM, unpublished results, June 1997), a second accessory protein recently has been cloned that does work with CRLR. The receptor activity-modifying protein (RAMP1) was cloned using the oocyte-CFTR assay, acts as a chaperone for CRLR, and is required for CRLR activation. The inability of RCP to function in cotransfection experiments with CRLR implies that RCP may work with a different receptor than CRLR. RCP is therefore a marker for this alternate CGRP receptor complex present in the ciliary body and iris blood vessels and ciliary epithelium and identifies a target for future studies on the pathophysiological action of CGRP in the eye.

Acknowledgments

The authors thank Lake Paul for his technical assistance in the cloning of the rabbit CGRP–RCP cDNA.

References


